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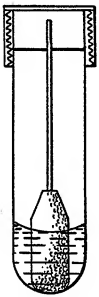
(54) Title: NON-INVASIVE SAMPLING METHOD FOR NUCLEIC ACID ANALYSIS

(57) Abstract

A method for obtaining biological material of an animal, suitable for conducting nucleic acid analysis thereon, is disclosed, comprising the steps of: (a) collecting by non-invasive means, from a site at a first location selected from the group consisting of exudate, body surface, and body cavity, a sample containing a first material; (b) modifying the first material so as to stabilize nucleic acids contained therein, by providing a second material at the first location; and (c) causing the stabilized nucleic acids to be delivered to a second location for performing nucleic acid analysis thereat.



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NON-INVASIVE SAMPLING METHOD FOR NUCLEIC ACID ANALYSIS

TECHNICAL FIELD

The invention concerns a method and device for obtaining nucleic acids from an animal suitable for genetic analysis.

BACKGROUND OF THE INVENTION

The analysis of the structure, organization and sequence of nucleic acid molecules is of profound importance to the prediction, diagnosis and treatment of human and animal disease, in forensics, in epidemiology and public health, and in the elucidation of the factors that control gene expression and development.

In one of the first examples of genetic analysis, James Wetherby established his famous equine studbook (1791) in which he recorded the characteristics of 80 foundation horses. This analysis was relatively crude relying on selected phenotypic characteristics of individual animals, such as coat coloring and markings of the animals. In the late 1970's, biochemical data obtained from blood samples was added to the record for each horse. Samples were collected from each animal to be registered and the results were matched to similarly typed parents to confirm the pedigree of the offspring. This form of biochemical analysis became known as blood typing. Although blood typing provides a more accurate record of pedigree than former methods of color and markings, several disadvantages are inherent in this approach, the primary disadvantage being the requirement of blood for the analysis. Not only do blood samples require invasive means of collection but blood is also a carrier of infectious diseases making it a potential health hazard during transport to a diagnostic laboratory. This health hazard is realized when spillage or leakage of the sample occurs. Blood has the further disadvantage of being adversely affected by storage at ambient temperatures. In some cases where either prolonged

transport times have occurred or the sample has been delayed in the initiation of mailing, the samples have already deteriorated by the time they arrive at the analytical laboratory and are unusable. Furthermore, the invasive collection of blood requires the services of a skilled individual usually a veterinarian to take the sample. This service adds to the cost of sampling and ultimately to the registration process. Even with skilled assistance, a timid, nervous or young animal can be injured during the process and there is a risk of infection at the sample site together with the risk of unsightly body reactions to the lesion formed by the needle.

Once blood samples have been collected, multiple diagnostic formats are applied to the sample in a panel of tests. These diagnostic formats commonly utilize reagents and protocols that are subject to interlaboratory variability. Furthermore, additions or improvements to the standard panel of tests are limited because of statistical incompatibility between the results from one set of animals tested using one approach and another set of animals tested differently.

There is a need therefore for alternative types of testing that do not depend on blood but can be performed on other biological material. In addition, it would be desirable to have a sampling method that is reliable, reproducible and safe to the animal, and associated with the method, a low cost device that can be used directly by unskilled individuals, the sample being in a form that can be subsequently safely transported to a diagnostic laboratory in a form that does not deteriorate over prolonged periods and that is protected from spillage and leakage.

Since 1791 when the thoroughbred registry was initiated, numerous registries have been formed for a wide range of horse breeds and also for other domesticated animals including dogs, cows, pigs and chickens. These registries have generally followed the approach of the

Thoroughbred registry by first using physical markers and then turning to blood typing. These registries have a similar need to that of the thoroughbred registry for alternative methods of sample collection that do not rely on blood.

The fundamental unit of information concerning any living organism is the nucleic acid contained within its cells that codes for every characteristic of the animal. In contrast to the phenotypic characteristics of an organism having the limitations described above, a test that measured genotypic characteristics of organisms by directly analyzing the nucleic acid itself would provide a reliable description of the organism and its pedigree.

A test of this type has been described in applicatior serial no 08/145,145 herein incorporated by reference in which a set of single nucleotide polymorphic DNA markers have been described that are capable of establishing the identity of an organism.

In order to characterize large numbers of subjects by their nucleic acid, a device and method is required, suitable for obtaining samples of nucleic acid that is simple, rapid, reliable and low cost and places samples in a form that facilitates their transportation and storage.

SUMMARY

The invention satisfies the above-stated needs. A novel sampling device and method is provided for obtaining a sample of nucleic acids for analysis from an animal for purposes including parentage determination.

In a preferred version of the invention, an improved method for obtaining biological material of an animal, suitable for conducting nucleic acid analysis thereon is provided. This method includes the following steps: collecting a sample containing a first material, by non-invasive means from a site at a first location selected from the group consisting of exudate, body surface, and body cavity; modifying the first material so as to stabilize nucleic acids contained therein, by providing a second

material at the first location; and causing the stabilized nucleic acids to be delivered to a second location for performing nucleic acid analysis thereat.

A further embodiment of the invention is a kit for obtaining a sample containing epithelial cells from an organism for nucleic acid analysis including means for obtaining the epithelial cells non-invasively; and a receptacle for receiving the epithelial cells, wherein the receptacle contains a means for modifying the cells so as to stabilize cellular nucleic acids.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a view of the receptacle containing a solution for stabilizing nucleic acids;

Figure 2 is a view of the sampling device; and

Figure 3 is a cross sectional view of the sample tube with sampling device in place;

Figure 4 is a cross section of horse's nostril opened laterally to expose the nasal diverticulum with a sampling device in place for collection of sample.

DETAILED DESCRIPTION OF THE INVENTION

"Site" is defined here and in the claims as the source of biological material obtained from the animal or plant.

"Location" is defined here and in the claims as the geographical placement of the animal, plant or diagnostic laboratory. The first and second location described here and in the claims are separated by a distance and a transport time.

The present invention is directed to a method and device for obtaining biological material, from an organism by non-invasive sampling for purposes of nucleic acid analysis, more particularly DNA analysis. A feature of the invention is the modification and stabilization of the sample at a first location and subsequent transport to a second location safely and without loss of sample through leakage or spillage.

According to the invention, the steps of the method are simple, and relatively safe to perform in the field. The

sampling device is low cost, does not require a medical expert and reproducibly provides sufficient material for subsequent DNA analysis and archiving of samples.

An example of a parentage test that does not rely on blood is that of analysis of the DNA of an animal or plant. DNA is present in every tissue of the organism so that in theory any tissue would be suitable for DNA analysis. According to the invention however, the choice of the site for obtaining a sample is determined by the accessibility of the site and the availability of sufficient material for analysis and archiving. This approach can be contrasted with methods of swabbing animals for infectious material analysis. The latter method of sampling is restricted to those sites at which infection occurs and depends upon the growth of the infectious agent.

According to the invention, the nose has been selected as the preferred site for the horse, because the equine nostrils are relatively accessible and contain a relatively large amount of readily available material suitable for nucleic acid analysis.

Differences between collecting samples for nucleic analysis from the nose and collecting samples for infectious disease diagnosis from the nose include the amount of the first material that is utilized in the diagnostic test and the characteristics of the second material. In order to determine the presence of an infectious agent in the nose of an animal, a swab is touched to an infected surface so as to pick up as little as a single microbe. The infectious agent is subsequently placed in a nutrient media (agar slope) to allow replication of the agent before, during and after transportation to a second location. In contrast, sample collection for nucleic acid analysis does not involve self replication. Instead, an amount of sample, sufficient for analysis, is placed in contact with a second material that modifies the cells and stabilizes the nucleic acids contained within the cells before transportation.

In contrast to samples for nucleic acid analysis, transportation of infectious material placed on solid nutrient agar do not present spillage or leakage problems although prevention of contamination by airborne microbial contaminants is necessary.

In the preferred embodiment of the invention, biological material is modified by lysing cells contained within so as to release the nucleic acids. The released nucleic acids are stabilized by means of buffers, chelating agents and antimicrobial agents at the first location prior to transport. The advantages of this approach include protection of the nucleic acids from degradation during transport and storage at ambient temperatures, and reduction in the risk of a health hazard associated with accidental release of unmodified biological material. A further advantage of this approach is that the first step of the analysis has been performed before the sample reaches the laboratory making further analysis more efficient.

Recent improvements in DNA analyses for parentage testing including automated microsatellite analysis (Fregeau C.J. and Fourney R.M. 1993, Biotechniques 15:100-119) and genetic bit analysis (U.S. Application Serial No. 07/664,837) as applied to single nucleotide polymorphisms. These methods have for the first time presented the need for suitable methods for collecting large numbers of samples from livestock and humans that are simple to use, safe, low cost and readily transportable and furthermore are in a form that is compatible with both the initial steps of DNA analysis conducted in the laboratory as well as for archiving of samples.

In a preferred embodiment, DNA analysis for parentage verification may be performed by determining the identity of each of a set of single nucleotide polymorphisms of a target animal, and the corresponding single nucleotide polymorphisms of a reference animal so as to establish whether the polymorphisms contain the same single nucleotide at their respective polymorphic sites and hence to obtain a

genotype for an individual. Methods of genotype determination have been described in patent application 07/775,786 herein incorporated by reference.

Comparison of the profile of polymorphic markers for an animal and its alleged parents provides a means to verify parentage of the animal with a high level of statistical accuracy.

Examples of genetic analysis using single nucleotide polymorphisms (SNPs) are provided in U.S. Application Serial No. 08/145,145. Statistical analysis of SNPs can be used for any of a variety of purposes. Where a particular animal has been previously tested, such testing can be used as a "fingerprint" with which to determine if a certain animal is, or is not that particular animal.

The preferred method of analyzing SNPs is by genetic bit analysis (GBA). This is a method for determining the identity of a nucleotide base at a specific position in a nucleic acid of interest. This method may be applied to parentage verification by selecting at least one polynucleotide GBA primer that is complementary to a nucleic acid sample from the animal. In an embodiment of the invention, the sample DNA may be amplified prior to hybridizing the animal DNA to the polynucleotide template. Methods of amplification may include one of: polymerase chain reaction (PCR) based amplification, ligase chain amplification or other ligase mediated reaction, self sustained sequence replication, strand displacement amplification or Qβ replication, a preferred embodiment being PCR. Following hybridization, template dependent extension of the primer in the presence of at least one dideoxynucleotide derivative is performed so as to incorporate the dideoxynucleotide derivative at a site complementary to the polymorphic nucleotide in the primer nucleic acid; and subsequently to determine the identity of the polymorphic nucleotide.

The ability to determine multiple SNPs for multiple animals rapidly and cost effectively includes an automated

test for which sampling devices and methods here described can be used.

An appropriate sampling device is described (Figs. 1-3) that is efficient at collecting biological material from an animal in an amount suitable for DNA analyses without causing undue irritation to the animal. Example 1 describes how the method and device of the invention is used to collect a sample of equine cells from the nostril. The method and device is different from the method described by Ellegren et al. 1992 (Animal Genetics 23:133-142) in which hairs were collected from individual horses for DNA analysis. That approach has disadvantages including contamination of the sample from one horse by the hair of another and difficulties in the handling of single hairs that may be easily misplaced during the initial stages of sample preparation. According to Ellegren et al. 1992, the hair samples were transported to a second location without any processing performed prior to reaching the laboratory. Furthermore, owing to the high protein/DNA ratio, less DNA is recovered from hair than is possible from a sampling device as described in Figure 1-3.

The method and device of the invention further differs from the approach taken by Richards et al. 1993, Human Molecular Genetics 2:159-163 in which cytology brushes and female dacron swabs were used to collect samples of cheek cells. The samples were allowed to dry on the sampling device and then shipped to the diagnostic laboratory for DNA analysis. DNA analysis was conducted directly from material obtained from the swab or brush in the laboratory by immersing the swab in 600µl of 50mM NaOH and heating to 95°C.

In contrast to the method of Richards et al., a higher quality and greater yield of the nucleic acids is obtainable according to a preferred embodiment of the invention, from epithelial cells that are lysed and modified to stabilize the nucleic acids at the location at which sample is

obtained and subsequently transported to a second location for analysis.

Method for Obtaining Biological Material of an Animal Subject by Non-invasive Means.

There are a number of sites on an animal from which a sample may be collected non-invasively. These sites include the body surface (skin or hair), body cavities (mouth, nostril, ears, anus, genital areas), and body exudates (mucus, glandular secretions). Samples from these sites can be obtained with varying degrees of ease and reproducibility, dependent on the accessibility and the properties of the site. In Example 1, biological material for DNA analysis is collected from the nostril of a horse. In this example, the nose has some preferred properties, including relatively easy access to the body cavity; a moist epithelium that yields abundant cells and mucus material; the availability of relatively large amounts of biological material on a consistent basis; protection from natural contamination by contact with other animals; and relatively small amounts of dirt or contaminating microorganism.

Biological material comprising a mixture of mucus and epithelial cells is obtained when a sampling means is extended into the horse nostril. Sampling is rapidly achieved by inserting, rotating and withdrawing a sampling means, causing the horse minimum discomfort or irritation of the nasal mucosa, and large amounts of cellular material providing DNA are obtained (Tables 1 and 2). Following the collection of the sample, the first material is modified so as to release nucleic acids contained inside cells by the addition of a detergent solution, the detergent being selected from non-ionic detergents such as Tween plus a chaotrophic salt or ionic detergents such as 0.5% SDS. Other methods of modifying cells so as to release nucleic acids that are suitable for disrupting cells may be used providing that the integrity of the DNA is not compromised.

The released nucleic acids are then prepared for delivery to a second location by first causing the nucleic acids to be stabilized at ambient temperature in a form that is readily recoverable for subsequent DNA analysis. In a preferred embodiment of the invention, the nucleic acids are stabilized in solution in the presence of 5 mM EDTA that serves both as an antimicrobial agent and a DNAase inhibitor. Alternatively, other agents that act singly as an antimicrobial agent such as sodium azide or as a DNAse inhibitor (sodium citrate) may be used. In an alternative embodiment of the invention, the nucleic acid may be stabilized by contacting the sample with an adsorbent material such as filter paper and then air dried.

Subsequent to stabilization of the sample at ambient temperatures, the nucleic acid sample is transported and delivered to a second location for analysis thereat. In a preferred embodiment, this step involves placing the sample in a receptacle of a size and shape suited for holding the sample. In an embodiment of the invention, the receptacle is a tube, the tube being of sufficient size to contain the sampling device and a solution. The solution serves to release and stabilize the nucleic acids. The tube is leak resistant, resistant to breakage, and can be easily handled by the individual collecting the sample. The tube is in a form that is suited for transport to a second site and is compatible in size and shape with handling methods at the site of DNA analysis.

An alternative embodiment includes the delivery of a sample containing released nucleic acid adsorbed onto a absorbent paper and air dried, the paper subsequently enclosed in an envelope containing standard materials for maintaining dryness of the sample.

Because samples are commonly collected under field conditions, the receptacle includes a protective feature that protects the sample against spillage. For example, the protective feature of a sampling device placed in a tube containing solution is the presence of a leak proof seal

that prevents solution and sample loss in the event of dropping the sample before a cap is secured.

The concentration of nucleic acid in a sample may be determined at the time of collection of the sample at the first site or prior to nucleic acid analysis at the second site. DNA quantification can be performed at the first site by the inclusion of a reagent in the sample solution that changes color according to the concentration of nucleic acid present in the sample. Alternatively, the color reagent may be included in the sampling means or in any second material , that is used to stabilize the nucleic acid.

Example 1 details one approach to measuring DNA concentration at the second site.

Sampling Kit for Obtaining Samples of Biological Material from the Nostril of a Horse

A sampling kit of the invention contains at least one sampling device, at least one receptacle and a transport package. In a preferred embodiment, the sampling kit includes two sampling devices - one for use and the second as a spare in case the first is dropped or contaminated; and one receptacle to place the sampling device therein.

In a preferred embodiment, the receptacle is a tube containing a solution and has means to prevent spillage and leakage.

A preferred embodiment of the invention is shown in Figure 2. The sampling means (6) has a bulb-shaped, abrasive, adsorptive surface formed from a biologically inert substance. Examples of biologically inert substances include plastic foam, cotton and brush material. The sampling means is supported on an extending means (7) formed from semi rigid material such as plastic (Hardwood products, Portland Me). The shape of the sampling means is tailored to the cavity from which the biological material is collected. For example, a sampling means having a length of about 2 cm and a width of about 1 cm is suited to collecting samples from the nasal cavity of the horse, where these dimensions are suited for a horse of any age. The extending

means is of a sufficient length (13 cm) for an operator to place the sampling means (2.9 cm) on the surface of the nasal diverticulum (8) (Fig. 4). The preferred length of the extending means is 15.9 centimeters although longer or shorter extending means could be used.

Although the preferred embodiment is a sampling means (6) on an extending means (7), another embodiment of the invention may include a sampling device placed on a digit, for example the thumb, of the person handling the animal, and the sample collected by inserting the sampling device on the digit into the nose so as to collect a sample.

Following collection of the sample, the sampling device is placed in a receptacle (Fig. 3), the receptacle (Fig. 1) being formed of an inert material, the material being sufficiently unbreakable to resist destruction by commercial transport. The shape of the receptacle should be suited to inserting and holding the entire sampling device. The receptacle may be labelled for purposes of identifying the sample.

In an embodiment of the invention, the receptacle is a tube (4) although other shaped receptacles may be used, the tube being made of polyethylene terphthalate (Encon Corp., Dayton, OH). Other material may be used including polystyrene, polypropylene, other plastics and glass. The tube has dimensions of 15.4 cm in length and 3 cm width, sufficient to hold the sampling means.

Contained within the tube is a solution (5) that provides a means of stabilizing the nucleic acids at ambient temperatures for extended periods of time. The solution contains reagents that inhibit the action of nucleases, chelate divalent cations and retard microbial growth. In a preferred embodiment, the solution has a volume of 5 ml and contains Tris buffer pH 8.0 in a concentration range of 1mM-1000mM, NaCl in a concentration range of 10 mM-1000 mM, SDS in the range of 0.05%-5% and EDTA in a concentration range of 0.1 mM-100 mM. Other buffers including sodium or potassium phosphate, MOPS, HEPES, TAPS, TOPS and biological

buffers may be used in place of TRIS. Other salts including KCl may be used in place of NaCl. Other chelating agents such as sodium citrate could be used in place of EDTA. Other ionic detergents including sodium deoxycholate and cetylpyridium chloride may be used in place of SDS. Non-ionic detergents such as Nonidet P40 and Triton X-100 may also be used, but must first be accompanied by a chaotropic salt such as guanidium HCL. An insoluble DNA binding reagent may also be included to initiate DNA purification en route.

In a further embodiment of the invention, a color indicator of DNA concentration is incorporated into either the transport medium, the sampling means or the tube containing the sampling means so as to provide a measure of the amount of total DNA contained within the biological sample.

Spillage prevention of the solution during transport avoids loss of the preservative solution and thereby optimizes the probability of the specimen arriving in analyzable form. The open end of the receptacle through which the sampling device is introduced in the site at which potential leakage may occur. In a preferred embodiment, a cup shaped piece of plastic such as low density polyethylene ("caplug" from Protective Closures, Buffalo, NY) (2) is fitted snugly into the opening of the tube (4). The plastic is scored and cut (3) so that it can be pierced with the sampling device (6). Other potential spillage prevention systems include a membrane or plug inside the tube which can be dislodged before use.

The tube containing the sample should be sealed during transit. The opening can be threaded (9) and a sealing cap used (1) to close the tube. In a preferred embodiment, the cap is made of plastic with an internal sealing liner (Polyseal Corp., Baltimore, MD). The part of the cap that contacts the solution should be biochemically inert (for example, polyethylene).

After placing the sample in the tube, the tube is transported in a transport package and delivered to a second site for analysis. The transport package should conform to postal regulations for the transport of biological specimens. In a preferred embodiment, the transport package is formed from high density paper with an interior made of bubble wrap, also containing a plastic enclosure for leakage protection. Further leakage protection can be introduced by adding any commercial product that adsorbs liquid. This form of packaging protects the sample from damaging insult and has secondary leakage protection.

Using the method of the invention, samples obtained from farms around the country were maintained for prolonged periods at ambient temperature without impairing the quality of the sample, prior to arrival at the laboratory for analysis.

Prior to initiating analysis, the nucleic acid may be precipitated and impurities removed. One method for precipitating the DNA in the sample is by adding guanidine HCL containing ethanol and subsequently filtering the mixture so as to retain the DNA contained in the precipitate on the filter. The DNA is redissolved in 800 µl of water and may subsequently be used for DNA analyses or for archiving.

Nucleic Acid Analysis

In an embodiment of the invention, the samples received by the laboratory are in a form suitable for performing of the first step of DNA analysis and for archiving the DNA without further modification. In another embodiment, the DNA may be precipitated and filtered prior to analysis so as to remove inhibitory substances and to prepare the DNA for DNA analysis and archiving.

Samples collected according to the methods and device of the invention may be analyzed for purposes of identity determination, trait analysis, and disease susceptibility in addition to parentage verification.

An example of sample analysis may include the determination of the identity of selected polymorphic nucleotides for purposes of genotype determination. In a preferred embodiment, a region of the sample DNA, containing an SNP is amplified using the polymerase chain reaction. The amplified material is rendered single stranded using the method described in US patent application 08/155,746 (herein incorporated by reference). These single stranded molecules are then hybridized to a GBA primer oligonucleotide that has been immobilized onto a solid surface. The immobilization would be performed using the method of US patent application 08/155,746 (herein incorporated by reference). The hybridized molecules are then incubated in the presence of one or more chain terminating nucleoside triphosphates under conditions sufficient to permit the incorporation of such a derivative onto the 3'-end of the primer as described in US patent application 07/775,786 (herein incorporated by reference). The identity of the polymorphic nucleotide may then confirmed by determined by a statistical analysis of numerical reaction values (U.S. patent application Serial No. XXX, herein incorporated by reference). The selection of informative single nucleotide polymorphisms has been described in detail in U.S. application No 08/145,145 herein incorporated by reference.

EXAMPLES

Example 1: Field testing of Non-invasive Sampling Method and Device in Horses for Nucleic Acid Analysis.

The sampling device was provided to the user in a sealed bag. In addition, a labelled tube having a bar code corresponding to the identity of the horse and containing a solution was also provided. A mailing envelope suitable for transporting the tube containing sampling device and sample was provided.

In a pilot study, the sampling device was provided to 14 horse farms in different geographic locations within the

United States. Some of the horses were restrained during the taking of a sample although restraint was not required for all animals. The handlers were first-time users. The sampling device was rapidly inserted into a nostril, rotated and removed. The sampling device was immediately placed in a pre-labelled tube containing a 5 ml solution of 10mM Tris, 100mM NaCl, 0.5% SDS and 5mM EDTA by pushing the sampling means through the slit on the caplug placed at the open end of the tube and then sealing the sampling device and solution in the tube with a screw cap.

The process was repeated with the second nostril. When all horses on the farm had been sampled, the tubes were transported in conventional padded mailing envelopes to the analytical laboratory and analyzed for DNA content.

At the laboratory, samples were analyzed by dot blot to estimate the total amount of DNA present. Aliquots (200 µl) of the swab transport solution containing stabilized nucleic acids were prepared for dot blot analysis by incubating proteinase K (100 µg/ml final conc) at 55°C for 2 hours followed by phenol extraction. These were filtered in duplicate onto nylon membranes in a vacuum manifold. A standard DNA concentration curve was prepared on each filter using purified equine DNA. The blots were then hybridized overnight to a ^{32}P-labelled equine total genomic probe (1.6 x 10^9 dpm/µg) prepared by random priming. DNA content was estimated by comparison to the standard curve of equine DNA on the same blot. Dot blot analysis confirmed that equine DNA is collected by nasal swabbing and is present in great enough quantity to perform PCR based DNA analyses.

The results are shown in Tables 1 and 2. DNA content of the samples obtained, ranged from 10µg to 1000 µg total DNA with a mean recovery rate of about 87.75µg/horse (Table 1) and a greater than 95% success rate in obtaining over 5µg of sample DNA/horse (Table 2).

TABLE 1

Florida Farm Statistics:
Based on Dot Blot Analysis

FARM	NO. SWABS	MEDIAN µ G
Good Chance	15	140
Hooper	15	100
Claude Ogle	11	60
Farnsworth	35	160
Kinsman	15	60
Marablue	15	87.5
Live Oak	15	45
Meadowbrook	15	90
Silverleaf	30	70
Hagemeyer	20	65

	Total Swabs	Average µ g	Median µ g
Overall:	186	87.75	87.5

TABLE 2

Farm Statistics:
Based on Dot Blot Analysis

FARM	NO. SWABS	NO. HORSES	% SWABS < 5 μG	% HORSES < 5 μG
A	82	41	0	0
B	60	30	0	0
C	50	25	2	0
D	50	25	18	0
E	36	18	5.6	0
F	30	15	33.3	0
G	20	10	5	0
H	18	9	0	0
I	10	5	10	0
J	68	34	11.8	2.9
K	50	25	12	4
L	46	23	6.5	4.3
M	60	30	18.3	10
N	50	25	32	12

Total Swabs	Total Horses	Overall % Swabs < 5 μg	Overall % Horses < 5 μg
630	315	7	2.9
		Median % Swabs < 5 μg	Median % Horses < 5 μg
		8.25	0

Although certain preferred embodiments of the present invention have been described, the spirit and scope of the invention is by no means restricted to what is described above.

What is claimed is:

1. A method for obtaining biological material of an animal, suitable for conducting nucleic acid analysis thereon, comprising:

(a) collecting by non-invasive means, from a site at a first location selected from the group consisting of exudate, body surface, and body cavity, a sample containing a first material;

(b) modifying the first material so as to stabilize nucleic acids contained therein, by providing a second material at the first location; and

(c) causing the stabilized nucleic acids to be delivered to a second location for performing nucleic acid analysis thereat.

2. A method according to claim 1, wherein the site is a body cavity, and the animal subject is a horse.

3. A method according to claim 2, wherein the body cavity is a nostril of the horse.

4. A method according to claim 3, wherein the biological material is a mixture of epithelial cells and mucus.

5. A method according to claim 1, wherein step (a) further comprises collecting the sample with a device, the device having a sampling means and an extending means, the sampling means having a shape and size suitable for contacting the site and an abrasive and adsorptive surface suitable for collecting the sample.

6. A method according to claim 5, wherein the sampling means is shaped in the form of an elongated bulb having a diameter of approximately 1 cm and a length of approximately 2 cm.

7. A method according to claim 5, wherein the handling means has a length of about 15 cm and is formed from a semiflexible material.

8. A method according to claim 1, wherein step (b) further comprises containing the second material in a receptacle suitable for transporting the stabilized nucleic acid to the second location.

9. A method according to claim 8, wherein the receptacle includes:

a tube formed from a plastic polymer having a length sufficient to contain the device, the tube having a closed end and an open end; and

means to restrict loss of solution from within the receptacle during sampling

10. A method according to claim 9, wherein the tube has a length of at least 15 centimeters and a diameter of at least 1.5 centimeter,

11. A method according to claim 9, wherein step (b) further comprises:

(i) preventing spillage of solution by means of a membrane containing a valve-like opening placed at the open end of the tube, for insertion of the sampling means therethrough, the valve-like opening gripping the extending means; and

(ii) preventing leakage of solution by means of a cap placed at the open end for sealing the tube.

12. A method according to claim 8, wherein the receptacle contains a colored reagent for determining the amount of nucleic acid contained within the sample.

13. A method according to claim 8, wherein the second material is a solution comprising a detergent for modifying the biological material so as to release nucleic acids.

14. A method according to claim 13, wherein the solution further comprises stabilizing agents, the stabilizing agents including an antimicrobial agent and a DNase inhibitor.

15. A method according to claim 13, wherein the solution contains a colored reagent for determining the amount of nucleic acid contained within the sample.

16. A method according to claim 1, further comprising an additional step (d) of performing parentage verification by nucleic acid analysis.

17. A method according to claim 1, further comprising an additional step (d) of performing identity determination by nucleic acid analysis.

18. A method according to claim 1, further comprising an additional step (d) of performing genetic trait analysis by nucleic acid analysis.

19. A method according to claim 1, further comprising an additional step (d) of performing disease susceptibility analysis by nucleic acid analysis.

20. A method according to claim 16, wherein the step (d) further comprises the step of performing nucleic acid analysis to identify polymorphic nucleotides including:

(i) hybridizing at least one strand of the sample nucleic acid or a derivative of the sample nucleic acid to at least one polynucleotide primer, each primer being complementary to a selected nonpolymorphic region of the sample nucleic acid, wherein the nonpolymorphic site is adjacent to a polymorphic region on the sample nucleic acid;

(ii) extending each polynucleotide primer by the addition of at least one dideoxynucleotide derivative, the dideoxynucleotide being complementary to the polymorphic site on the sample nucleic acid; and

(iii) identifying the polymorphism.

21. A method according to claim 20, wherein the polymorphic site is a single nucleotide polymorphism.

22. A method according to claim 20, wherein step (d) of parentage verification further comprises the steps of;

(e) determining, for a single nucleotide polymorphism of a target animal, and for a corresponding single nucleotide polymorphism of a reference horse, whether the polymorphisms contain the same single nucleotide at their respective polymorphic sites; and

(f) repeating step (a) for a plurality of single nucleotide polymorphisms to obtain a genotype for the animal;

(g) comparing the genotype for the animal with that of its alleged parents; and

(h) verifying parentage of the animal from the comparison of the genotypes.

23. A method according to claim 17, wherein step (d) of identity determination further comprises the steps of;

(e) determining for a single nucleotide polymorphism of a target animal, and for a corresponding single nucleotide polymorphism of a reference horse, whether the polymorphisms contain the same nucleotide at their respective polymorphic sites,

(f) repeating step (a) for a plurality of single nucleotide polymorphisms,

(g) obtaining a genotype of the animal; and

(h) determining the identity of the animal from the genotype.

24. A method according to claim 1, wherein the step (d) further comprises the step of performing nucleic acid analysis to identify polymorphic nucleotides by the additional steps of:

(i) hybridizing at least one strand of the sample nucleic acid or derivative of the sample nucleic acid to at least one polynucleotide primer, each primer being complementary to a selected nonpolymorphic region of the sample nucleic acid, wherein the nonpolymorphic site is adjacent to a polymorphic region on the sample nucleic acid;

(ii) extending each polynucleotide primer by the addition of at least one dideoxynucleotide derivative, the dideoxynucleotide being complementary to the polymorphic site on the sample nucleic acid; and

(iii) identifying the polymorphism.

25. A method according to claim 24, wherein the polymorphic site comprises a single polymorphic nucleotide.

26. A method according to claim 24, wherein the step of immobilizing the polynucleotide primer precedes step (i).

27. A kit for obtaining a sample containing epithelial cells from an organism for nucleic acid analysis comprising:

(i) means for obtaining the epithelial cells non-invasively; and

(ii) a receptacle for receiving the epithelial cells, wherein the receptacle contains chemical means for modifying the cells so as to stabilize the cellular nucleic acids.

28. A kit according to claim 27, wherein the means for obtaining the epithelial cells includes a sampling means attached to an extending means.

29. A kit according to claim 28, wherein the sampling means has a size and shape suited for contacting a mucosal surface within a nostril of an animal.

30. A kit according to claim 27, wherein the sampling means has an abrasive adsorptive surface for collecting the epithelial cells.

31. A kit according to claim 30, wherein the sampling means has a shape selected from the group consisting of a bulb, a rod, an arrow and a mushroom.

32. A kit according to claim 31, wherein the sampling means is shaped in the form of an elongated bulb having a diameter of approximately 1 cm and a length of approximately 2 cm.

33. A kit according to claim 32, wherein the sampling means comprises a polymer wherein the polymer is compressible.

34. A kit according to claim 28, wherein the extending means is attached to the sampling means for positioning the sampling means in contact with the site and removing it therefrom.

35. A kit according to claim 34, wherein the extending means has a length of about 13 cm and is formed from a semiflexible material.

36. A kit according to claim 35, wherein the means for obtaining the epithelial cells includes an adsorbent abrasive material fitted over a digit on a hand of a human operator.

37. A kit according to claim 27, wherein the receptacle is of sufficient size to place the cells in contact with the modifying material.

38. A kit according to claim 37, wherein the modifying material is a solution containing a detergent and a stabilizing agent.

39. A kit according to claim 38, wherein the detergent is SDS and the stabilizing agent is EDTA.

40. A kit according to claim 27, wherein the receptacle contains means to prevent leakage out of the receptacle.

41. A kit according to claim 40, wherein the receptacle further comprises:

(i) a tube formed from a plastic polymer having a length of at least 15 centimeters and a diameter of at least 1.5 centimeter, the tube having a closed end and an open end; and

(ii) a membrane containing an opening for insertion of the sampling means therethrough, the opening forming a seal around the extending means, the membrane being placed at the open end of the tube.

42. A kit according to claim 41, further comprising a cap placed at the open end of the tube.

43. A kit according to claim 27, further comprising a

reagent contained within the receptacle, wherein the reagent changes color according to the concentration of the nucleic acids contained within the sample of biological material.

44. A kit according to claim 27, wherein the means for obtaining epithelial cells contains a colored reagent for determining the amount of the epithelial cells.

45. A kit according to claim 27, further comprising a bar code placed on the exterior of the receptacle for identifying the source of the sample.

46. A kit according to claim 28, further comprising a bar code placed on the extending means for identifying the source of the sample.

47. A kit according to claim 27, further including a transport package for holding the receptacle, the transport package being suitable for commercial transportation.

48. A kit according to claim 47, wherein the transport package is formed from paper and contains a plastic enclosure within.

49. A kit according to claim 27, wherein the receptacle is a filter paper containing the modifying material.

50. A kit according to claim 27, for collecting a sample containing sufficient nucleic for performing analysis of single nucleotide polymorphisms, wherein the analysis includes; hybridizing at least one strand of the sample nucleic acid or a derivative of the sample nucleic acid to at least one polynucleotide primer, each primer being complementary to a selected nonpolymorphic region of the sample nucleic acid, wherein the nonpolymorphic region is adjacent to a polymorphic region; and extending each polynucleotide primer by incorporation of at least one dideoxynucleotide derivative that is complementary to the polymorphic site on the sample nucleic acid so as to identify the polymorphism.

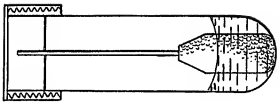

FIG. 3

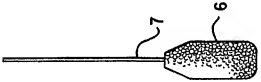

FIG. 2

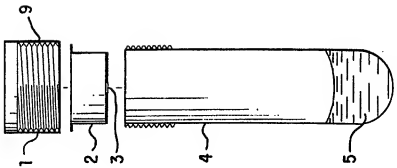

FIG. 1

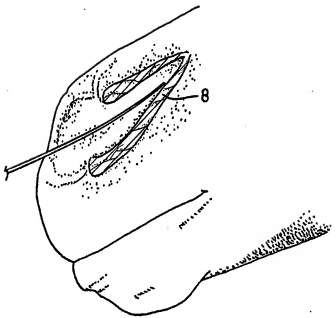

FIG. 4

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 95/00429

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G01N33/483 A61B10/00 B01L3/00 C12M1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 770 853 (BERNSTEIN) 13 September 1988 see the whole document ---	1,5-9, 12,15, 27-37, 39, 41-44,49
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 June 1995	29. 06. 95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Klein, C

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